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Physiological responses of estuarine phytoplankton to ultraviolet light-induced fluoranthene toxicity

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are common pollutants associated with urbanization and suburbanization in estuarine systems, but little is known about their effects on the physiological properties of microalgae. We examined the effects of ultraviolet (UV)-activated fluoranthene toxicity on (a) the growth, chlorophyll *a* content cell^{-1}, and pigment composition of axenic *Ankistrodesmus* sp. (an estuarine benthic green microalga) and (b) the phytoplankton population growth and pigment composition of natural communities from an urbanized (Murrells Inlet) vs. forested (North Inlet) salt marsh estuary. The zeaxanthin/violaxanthin ratio increased in *Ankistrodesmus* sp. cultures exposed to UV light in the presence of fluoranthene, supporting the hypothesis that xanthophyll cycling is an energy dissipative response to photoinduced PAH toxicity in this species. Exposure of natural communities to the combination of UV light and fluoranthene resulted in decreased chlorophyll production and increased zeaxanthin violaxanthin^{-1} in samples from the urbanized estuary (Murrells Inlet), but not North Inlet, suggesting that phytoplankton in the former ''fluoranthene-impacted'' estuary were more susceptible to fluoranthene toxicity. Consideration of xanthophyll cycling as a microalgal response to UV-activated PAH toxicity has implications to understanding the influence of these contaminants on microbial food web structure and ecosystem production.

Keywords: *Ankistrodesmus*; Benthic microalgae; Fluoranthene; Polyaromatic hydrocarbons; Urbanization; UV-induced toxicity; Xanthophyll cycle

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are compounds composed of two or more fused benzene rings that can adversely affect terrestrial (Boese et al., 1998), aquatic (Ren et al., 1996), and estuarine ecosystems (Lotufo, 1998). Although PAHs can originate from natural sources (e.g. forest fires), they are mainly derived from anthropogenic sources such as fossil fuel combustion, oil spills, or industrial processes (Ireland and Burton, 1996). Therefore, areas impacted by urbanized (e.g. industrial plants and commercial development) or suburbanized (e.g. residential development and automobile traffic) development are particularly susceptible to PAH pollution. Studies of the coastal zone of the southeastern United States have shown that total PAH concentrations are significantly higher in the sediments and water column of estuaries in developed locations than in undeveloped areas (Siewicki, 1995) and that fluoranthene consistently comprises the highest or second highest fraction of total PAHs in these locations (Fortner et al., 1996).

Studies of UV-induced PAH toxicity have focused on higher organisms and zooplankton (e.g. Weinstein et al., 1997; Boese et al., 1998), while studies on microalgae are predominantly based on freshwater species (e.g. Bastian and Toetz, 1982; Gala and Giesy, 1992, 1993, 1994). Direct toxic effects of fluoranthene and other PAHs on plants and animals have been demonstrated (Ren et al., 1996; Chandler et al., 1997; Boese et al., 1998; Lotufo, 1998), but toxicity can be substantially enhanced by exposure to ultraviolet (UV) radiation (Boese et al., 1998). Photoinduced toxicity has been detected in natural surface waters, runoff, and industrial effluent at concentrations below the aqueous solubility limits of PAHs (Gala and Giesy, 1992). PAH toxicity is known to be dependent on the intensity and duration of UV exposure. Evidence suggests that the photodynamic nature of PAH toxicity is derived from the compounds' abilities to absorb energy from UV light, which results in the production of excited singlet and triplet state molecules. The excited PAH molecules release energy through non-radiative pathways by generating singlet oxygen and other reactive oxygen species. Singlet oxygen is a powerful oxidant and can result in toxicity by oxidative stress in exposed biological tissues (Weinstein et al., 1997).

Carotenoid production has been demonstrated as a mechanism for protection against oxygen radical damage in higher plants and algae, though more commonly in response to stressful high light conditions (Porra et al., 1997) than UV-induced toxicity (Gala and Giesy, 1993). β-carotene is considered the major protective pigment in photosynthetic organisms based on its high ability to quench singlet oxygen, a function of the molecule's high number of conjugated carbon–carbon double bonds. Gala and Giesy (1993) investigated the ability of carotenoid pigments to protect *Selenastrum capricornutum* from the photoinduced toxicity of anthracene, a three-ringed PAH. Their study used fluridone, a pesticide that inhibits the biosynthesis of β-carotene and other carotenoids in algae and higher plants, to produce algal cells with different levels of carotenoids. The percent inhibition of ^{14}C-bicarbonate incorporation was found to be inversely related to colored carotenoid pigment concentrations in algal cells exposed to UV-induced anthracene toxicity. The authors concluded that capacity for protection against UV-activated anthracene toxicity was dependent on cellular carotenoid (including β-carotene) content in *S. capricornutum*.

In addition, or alternatively, to modifying cellular β-carotene content, higher plants and algae have evolved other energy dissipative mechanisms that involve adjustments in carotenoid content and composition. For example, the xanthophyll cycle is a radiationless method for energy dissipation that has been demonstrated as a response to photoinhibition in higher plants, some macroalgae, chromophytes, and chlorophytes (Falkowski and Raven, 1997). One type of xanthophyll cycle, termed the "violaxanthin cycle", involves reversible epoxidation of zeaxanthin to violaxanthin. When light absorption exceeds the light used for photosynthesis, proton accumulation (acidification) occurs in the thylakoid lumen and violaxanthin de-epoxidase is activated (Porra et al., 1997), resulting in a net increase in zeaxanthin (Goodwin, 1980). The role of the violaxanthin cycle in energy quenching is based on the increase in the number of conjugated carbon–carbon double bonds that occurs upon the de-epoxidation of violaxanthin to zeaxanthin, suggesting a decrease in energy state upon transformation. Zeaxanthin is assumed to be at a lower energy state than the lowest excited chlorophyll state and, therefore, is capable of accepting singlet energy from chlorophyll *a*. Light is absorbed by this process but excitation energy is not transferred to the reaction centers. In contrast to zeaxanthin, violaxanthin transfers energy to chlorophyll in the light harvesting process.

Similar to the response to photoproduction of oxygen radicals, it is possible that zeaxanthin may be capable of accepting singlet energy produced by photo-activated PAHs, in which case, the ratio of zeaxanthin to violaxanthin would be expected to increase in response to UV-induced PAH toxicity. To our knowledge, the operation of the violaxanthin cycle in response to UV-activated fluoranthene toxicity has not been examined. This study examined the physiological responses of an estuarine benthic green alga (*Ankistrodesmus* sp.) and the pigment responses of natural phytoplankton communities to UV-induced fluoranthene toxicity. We tested the hypotheses that: (a) estuarine microalgae respond to UV-induced fluoranthene toxicity by increasing β-

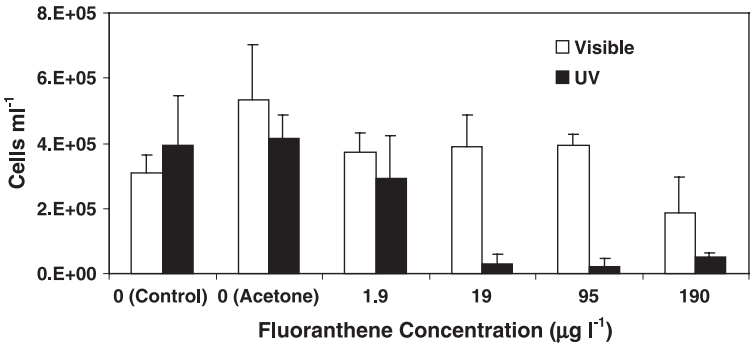

Fig. 1. Mean and standard deviation (error bars) of *Ankistrodesmus* abundance (cells ml^{-1}) at T_{36} for Control, Acetone, or Fluoranthene treatments under visible light or UV light. Data is from Southerland (2001), and follows the present study's protocol for culture experiments except that the acclimation and experimental irradiance level for visible light was 100 µE m^{-2} s^{-1}.

carotene cell^{-1} and (b) estuarine microalgae capable of violaxanthin cycling respond to UV-induced fluoranthene toxicity by increasing the cellular ratio of zeaxanthin to violaxanthin.

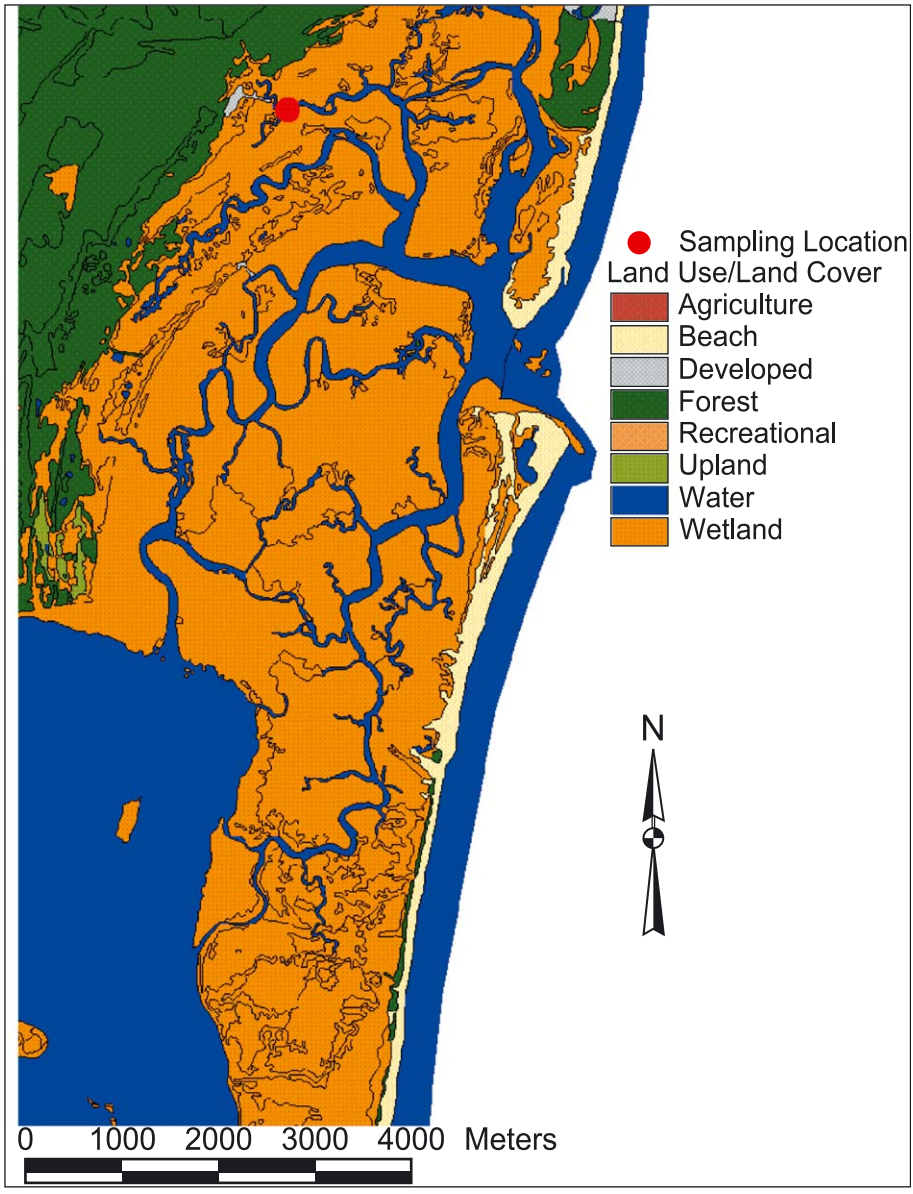

Fig. 2. North Inlet estuary, with sampling site location (red circle is NI 1) and land use/land cover features (Source: Baruch Institute GIP Laboratory). The "developed" area west of study site is the Baruch Marine Field Laboratory, a research institute.

2. Materials and methods

2.1. Culture conditions

Axenic monoclonal cultures of *Ankistrodesmus* sp. HP9101 (formerly called *Closterium* sp. HP9101, Lewitus and Kana, 1994) were used. *Ankistrodesmus* sp. is a benthic chlorophyte isolated by AJL from water collected in early spring 1991 from the Choptank River (salinity 7–12‰, temperature 15 °C), a subestuary of Chesapeake Bay (Lewitus and Kana, 1994), and routinely maintained in f/2-enriched seawater medium (Guillard, 1975) at 17‰ salinity and 23 °C. Cultures were acclimated to growth in 17‰ artificial seawater

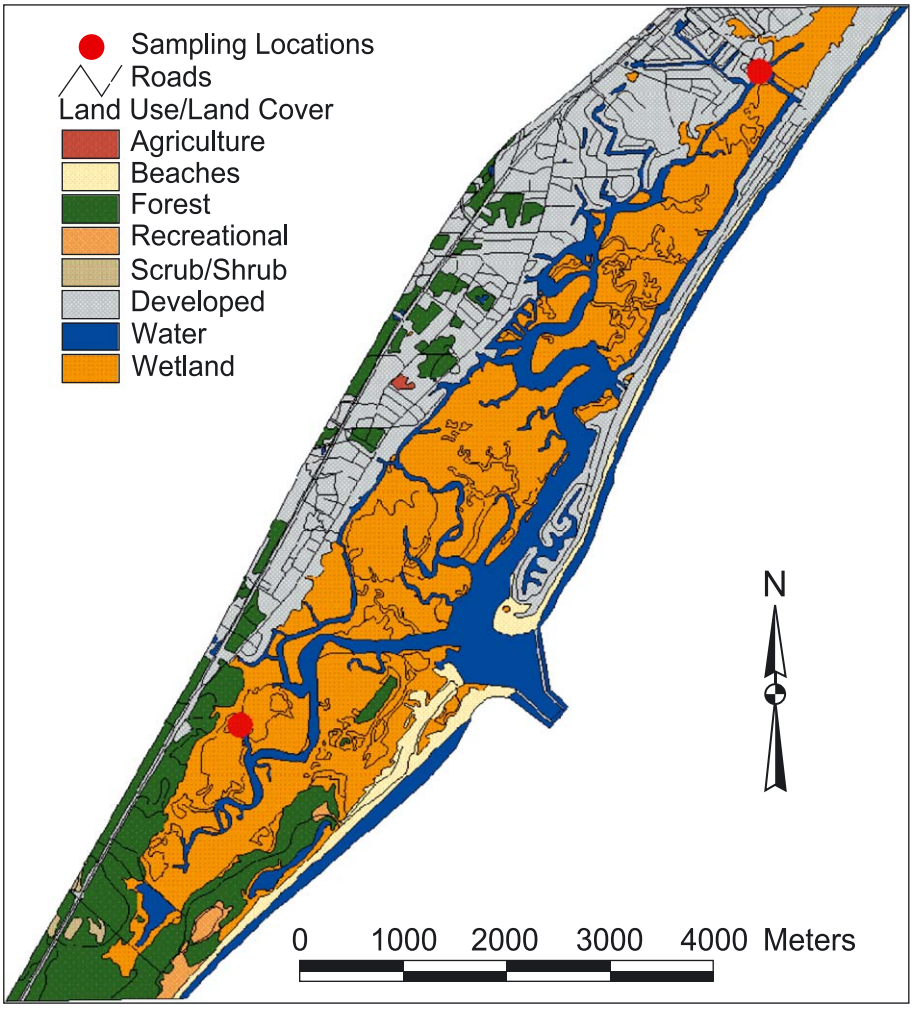

Fig. 3. Murrells Inlet estuary, with sampling locations (northern red circle is MI 2, southern red circle is MI 6) and land use/land cover features (source: Baruch Institute GIP Laboratory).

medium (Guillard, 1975, modified by Lewitus and Kana, 1994) at the appropriate experimental irradiance and temperature by repeated transfers from mid-exponential growth phase. Acclimation was determined when the specific growth rate of three successive transfers did not vary significantly (Brand et al., 1981). *Ankistrodesmus* sp. and other green algae contain violaxanthin and zeaxanthin (Porra et al., 1997), components of the xanthophyll cycle examined in this study.

2.2. Culture experiment

The time-course response of growth and pigment composition of *Ankistrodesmus* sp. to UV and fluoranthene exposure was examined. The fluoranthene concentration for this experiment (19 μg l^{-1}) was chosen based on preliminary experiments demonstrating that this was the lowest effective UV-activated concentration, within the range tested (1.9, 19, 95, and 190 μg l^{-1}), that caused an inhibitory growth response in *Ankistrodesmus* (Fig. 1). The 24-h EC_{50} calculated (Hamilton et al., 1977) at that concentration was 11.46 μg l^{-1} (Southerland, 2001). *Ankistrodesmus* sp. cultures in 17‰ artificial seawater acclimated to growth at ~75 μE m^{-2} s^{-1} were transferred during exponential growth phase to 1-l Erlenmeyer flasks, and incubated in an environmental chamber (see below) under the following culture media treatments: no addition artificial seawater control, 250 μM acetone

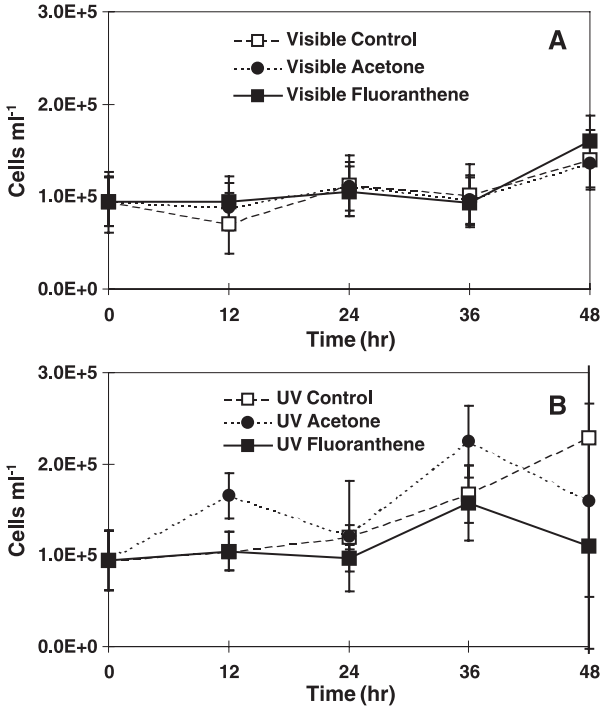

Fig. 4. Mean and standard deviation (error bars) of *Ankistrodesmus* abundance (cells ml^{-1}) vs. time for Control, Acetone, or Fluoranthene treatments under (A) visible light or (B) UV light.

addition (as carrier controls), or 19 μg l^{-1} fluoranthene. Fluoranthene primary stock was prepared by dissolving 53.4 mg of fluoranthene (Sigma, 99% purity) in 40 ml HPLC grade acetone. A diluted working stock was prepared by sterile-filtering (0.2 μm) 0.4 ml of this mixture into 40 ml of autoclaved de-ionized ultrapure water. Working stock was added immediately to culture media to achieve the desired concentration. Toxicant concentrations were not refreshed or renewed after the initial inoculation. Acetone working stock (1%) for carrier controls was prepared by adding 0.4 ml of HPLC grade acetone to 40 ml of autoclaved ultrapure water.

Triplicate flasks of each treatment were exposed to visible light (~ 75 μE m^{-2} s^{-1}) or a combination of visible and UV-A light (~ 805 μW cm^{-2}). Previous research with this species indicated that a UV-A intensity of 785 μW cm^{-2} is sufficient to cause growth inhibition in the presence of 19 μg l^{-1} fluoranthene, but does not result in a significant reduction in cell abundance in the absence of fluoranthene (Southerland, 2001). Initially (T_0), cultures were placed in the dark for 12 h to allow the test organisms to assimilate the fluoranthene prior to UV activation, after which the lights were turned on (T_{12}) and remained on for the duration of the experiment, 48 h. At 12-h intervals, T_{24}, T_{36}, and T_{48}, samples were fixed (2% glutaraldehyde) for cell counts, and filtered (GF/F) and frozen (− 80 °C) for determination of pigment composition by high performance liquid chromatographic (HPLC) analysis. Data are presented for a subset of pigments measured from *Ankistrodesmus* cultures. Data on neoxanthin, lutein, and

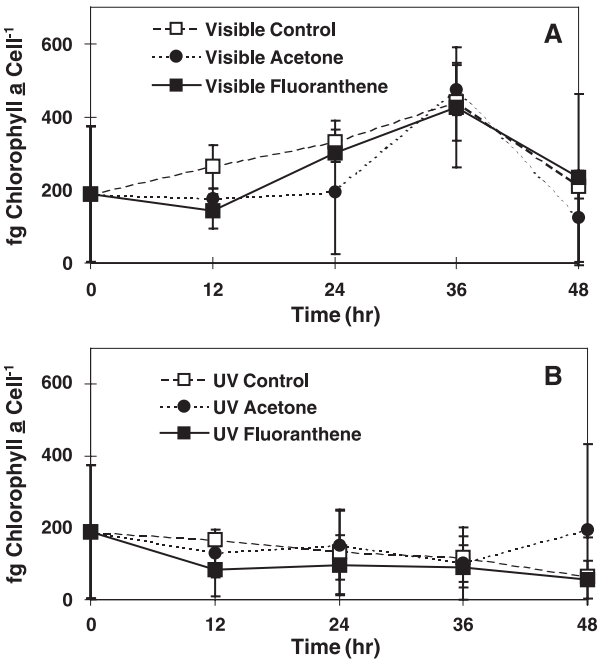

Fig. 5. Mean and standard deviation (error bars) of fg chlorophyll *a* $cell^{-1}$ vs. time for *Ankistrodesmus* cultures in Control, Acetone, or Fluoranthene treatments under (A) visible light or (B) UV light.

chlorophyll *b* are not shown because cellular content of these pigments did not vary with treatment.

2.3. Natural community experiment

Another experiment examined the effects of UV-activated fluoranthene toxicity on chlorophyll *a*, carotene (total of α- and β-carotene), violaxanthin, and zeaxanthinin concentration in natural communities collected from two South Carolina high salinity salt marsh estuaries. North Inlet (Fig. 2), near Georgetown, is considered a pristine forested estuary (a National Estuarine Research Reserve site), and is characterized by a lack of anthropogenic influence (Lewitus et al., 1998). Murrells Inlet (Fig. 3), located ~32 km north of North Inlet, resembles North Inlet in topography and tidal regime, but is impacted by suburbanized development in support of residential and tourist industries (Vernberg et al., 1992). Water samples were collected from 1 m below the surface during slack low tide from three locations. The NI 1 site is located at Oyster Landing in North Inlet adjacent to the Baruch Marine Field Laboratory (Fig. 2). MI 6 is located at the public boat landing in the southern, less developed part of Murrells Inlet, and MI 2 is located near a more developed section of Murrells Inlet, adjacent to residences, rental

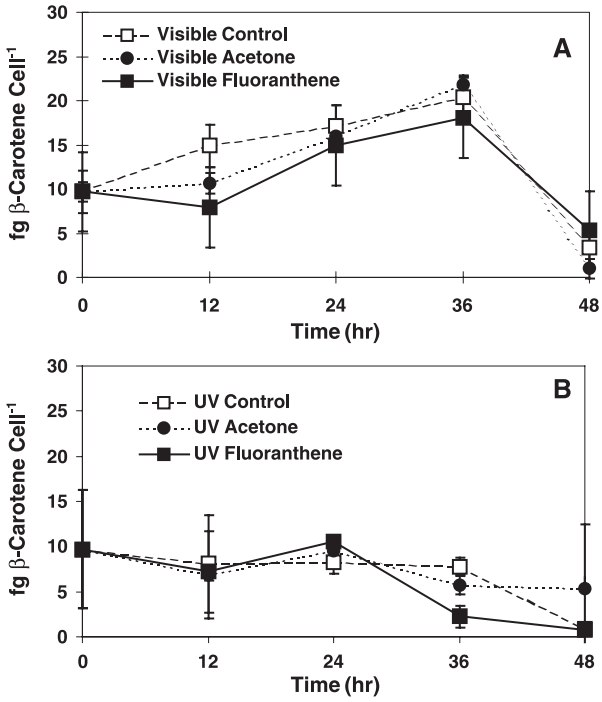

Fig. 6. Mean and standard deviation (error bars) of fg β-carotene cell^{-1} vs. time for *Ankistrodesmus* cultures in Control, Acetone, or Fluoranthene treatments under (A) visible light or (B) UV light.

properties, restaurants, and tourist-related industries, and is subject to a high volume of automobile traffic (Fig. 3). MI 6 is considered to be minimally impacted by urbanized practices, as reflected by the relatively low sediment PAH and fluoranthene concentrations in comparison, for example, to MI 2 (Siewicki, 1995). Mean sediment fluoranthene concentrations measured at MI 2 (e.g. 329 ng g dry wt^{-1}) were generally much higher than those measured at MI 6 (e.g. 46 ng g dry wt^{-1}), and still lower mean concentrations (e.g. 9 ng g dry wt^{-1}) were found at the North Inlet site (Siewicki, 1995; D. Bearden, unpublished data).

For the natural community experiments, a higher fluoranthene concentration (95 μg l^{-1}) was used in order to include the potential responses of phytoplankton less sensitive to this toxicant than *Ankistrodesmus*. Treatments consisted of no addition controls, acetone addition (1.25 mM) as carrier controls or 95 μg l^{-1} fluoranthene addition. The experimental protocol followed that of the *Ankistrodesmus* culture experiments except that UV-A light was supplied at ~800 μW cm^{-2}, and samples initially (T_0) placed in the dark with fluoranthene were afterward placed on a 12/12 h light/dark cycle for a total of 72 h (T_{72}). At T_0 and T_{72}, samples were filtered (GF/F) and frozen (−80 °C) for HPLC analysis.

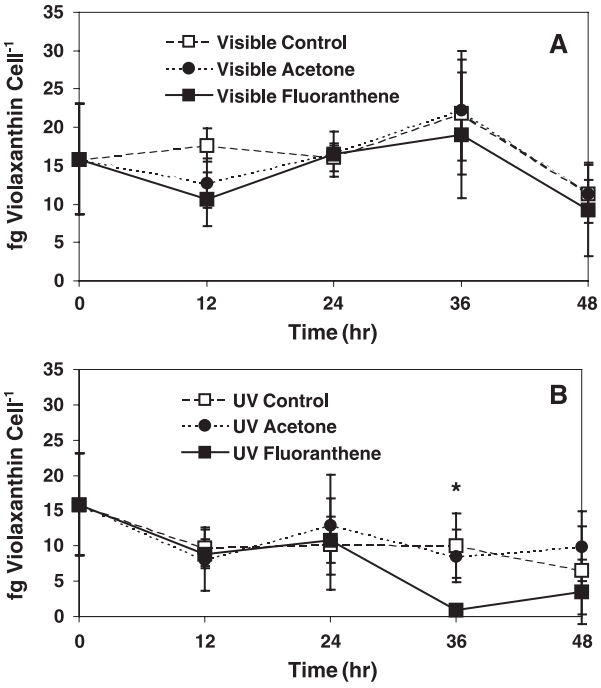

Fig. 7. Mean and standard deviation (error bars) of fg violaxanthin $cell^{-1}$ vs. time for *Ankistrodesmus* cultures in Control, Acetone, or Fluoranthene treatments under (A) visible light or (B) UV light. * indicates significant difference between Fluroanthene and other treatments.

2.4. Environmental chamber

Experiments were conducted in a Plexiglas® environmental chamber designed and constructed for the project. The approximate dimensions were 122-cm long, 91-cm deep, and 61-cm high. Lighting consisted of visible light (400–700 nm) and UV-A (320–400 nm). Visible light was provided by 40 W General Electric cool white fluorescent bulbs, and UV-A illumination was provided by 15 W Spectroline (Spectronics, Westbury, NY) bulbs. The visible source was located outside the Plexiglas® hood, which eliminated UV wavelengths less than 390 nm. The UV-A bulbs were selected because they were integrally filtered to produce only UV-A (at a peak of 365 nm), and not UV-B (280–320 nm) or UV-C (210–280 nm) light. Visible light intensity was quantified using a Biospherical Instruments Model 100 QSL photometer and UV-A light intensity was quantified by a Blak-Ray Model J221 (UVP, Upland, CA) long-wave ultraviolet measuring meter. Visible and UV-A treatments were separated into sections by partitions, and multiple light measurements were taken at shaker table height with no samples present in a grid pattern for each section. Visible and UV-A intensities were averaged and reported as the mean of those values. Visible light intensities were

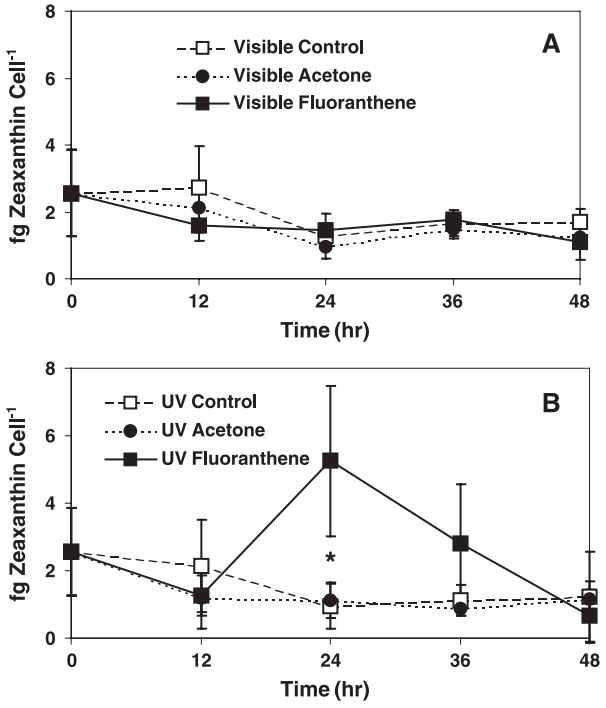

Fig. 8. Mean and standard deviation (error bars) of fg zeaxanthin cell^{-1} vs. time for *Ankistrodesmus* cultures in Control, Acetone, or Fluoranthene treatments under (A) visible light or (B) UV light. * indicates significant difference between Fluroanthene and other treatments.

adjusted, as necessary, with the use of neutral density screening. Replicates for each treatment were randomized within each light group to reduce bias due to factors such as uneven light distribution. Culture flasks were agitated continuously using a shaker table. Temperature in the environmental chamber was maintained at 26 °C (± 1 °C) for each experiment.

2.5. Analytical methods

Ankistrodesmus abundance (cell ml^{-1}) was determined microscopically using a hemacytometer. A minimum of 200 cells was counted for each replicate using either a Hausser Scientific 0.5 mm counting chamber (for cell densities $< 10^4$ cell ml^{-1}), or a Hausser Scientific 0.1 mm hemacytometer (for cell densities $>10^4$ cells ml^{-1}); Lewitus et al. (1998).

For HPLC analysis, algal cells were collected by filtration on Whatman GF/F glass fiber filters, placed in foil envelopes, and stored at − 80 °C until extraction. Pigments were extracted in 100% acetone using a tissue grinder. The slurry was then filtered through a Teflon HPLC syringe cartridge filter (0.45 μm) containing a glass-fiber prefilter. Pigment analysis was conducted using a Beckman System Gold HPLC and

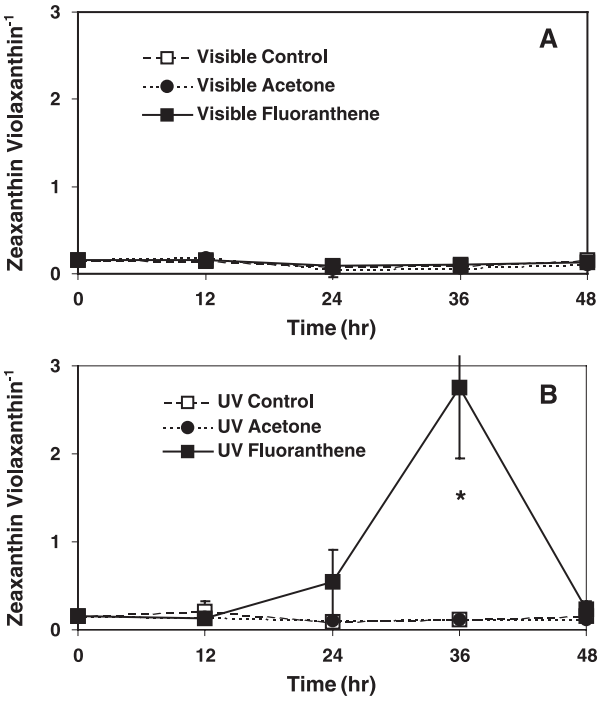

Fig. 9. Mean and standard deviation (error bars) of zeaxanthin violaxanthin^{-1} vs. time for *Ankistrodesmus* cultures in Control, Acetone, or Fluoranthene treatments under (A) visible light or (B) UV light. * indicates significant difference between Fluroanthene and other treatments.

System Gold V810 software with an external column heater, 125 solvent module dual pump, and photodiode array detector with deuterium lamp (monitoring 450 nm). The protocol is a modification of Van Heukelem et al. (1992, 1994), and uses temperature-control and a polymeric column. Pigment standards were obtained from L. Van Heukelem (Horn Point Laboratory, University of Maryland). Our method does not separate β-carotene from α-carotene. The concentrations of β-carotene reported here for *Ankistrodesmus* likely contain a minute quantity of α-carotene (< 10% of β-carotene; Porra et al., 1997).

In a separate experiment, we compared the effects of different freezing protocols on *Ankistrodesmus* pigment composition. To test the possibility that xanthophyll cycle reactions were proceeding during the interval between filtering and complete freezing of the sample, we compared the freezing method used in this study (i.e. placing foil envelopes immediately in the −80 °C freezer) with more rapid freezing (i.e. immersing foil envelopes immediately in liquid nitrogen). *Ankistrodesmus* cultures were grown under the protocol described above (Culture Experiment) except that acetone controls were not compared. No significant differences in violaxanthin cell^{-1}, zeaxanthin cell^{-1}, violaxanthin chlorophyll a^{-1}, zeaxanthin chlorophyll a^{-1}, or zeaxanthin violaxanthin^{-1} were found between freezing proto-

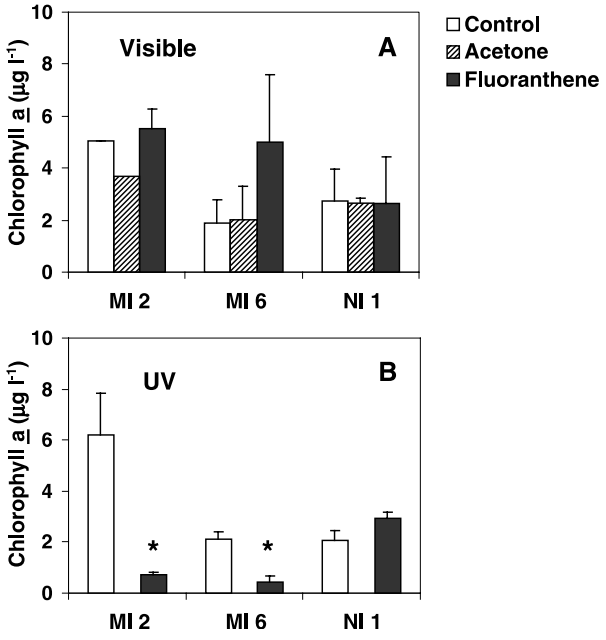

Fig. 10. Mean and standard deviation (error bars) of chlorophyll *a* concentration (µg l^{-1}) in water samples collected from Murrells Inlet sites 2 (MI 2) or 6 (MI 6), or North Inlet site 1 (NI 1), and incubated with (A) no additions (Control), acetone, or fluoranthene under visible light or (B) no additions (Control), or fluoranthene under UV light. * indicates significant difference between Fluroanthene and Control treatments.

cols in any treatment, and treatment differences between freezing protocols averaged 1% (normalized to cell abundance) or 4% (normalized to chlorophyll *a*); data not shown.

2.6. Statistical methods

An analysis of variance (ANOVA) was performed for each toxicity endpoint measured (e.g. β-carotene cell^{-1}, zeaxanthin cell^{-1}, violaxanthin cell^{-1}, zeaxanthin violaxanthin^{-1}) for each experiment to determine if a significant response was observed. UV controls were compared to visible light controls to detect differences in response due to UV exposure alone. Visible fluoranthene and acetone carrier treatments were compared to visible light controls, and to each other, to detect any variability between treatments that were not exposed to UV-A radiation. UV + fluoranthene treatments were then compared to the UV + acetone treatments to determine the effect of UV exposure on fluoranthene toxicity. Means comparisons employed the Wilcoxon/Kruskal–Wallis test when toxicity endpoints were not normally distributed. A repeated measures ANOVA was used to compare means because replicates were sampled over time (Kleinbaum et al., 1998). All statistical analyses were performed

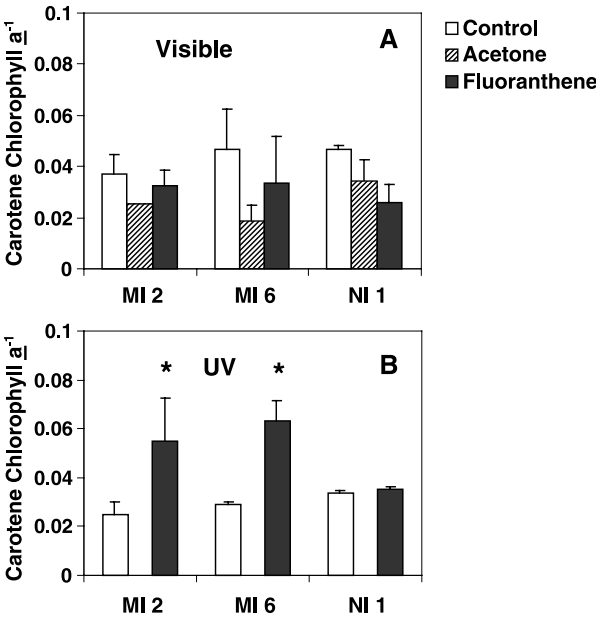

Fig. 11. Mean and standard deviation (error bars) of carotene chlorophyll a^{-1} in water samples collected from Murrells Inlet sites 2 (MI 2) or 6 (MI 6), or North Inlet site 1 (NI 1), and incubated with (A) no additions (Control), acetone, or fluoranthene under visible light or (B) no additions (Control), or fluoranthene under UV light. * indicates significant difference between Fluroanthene and Control treatments.

using SAS® and JMP® software (Sall and Lehman, 1996). The significance level for all experiments was set at $\alpha = 0.05$.

3. Results

3.1. Ankistrodesmus

Mean cell abundance (Fig. 4) or chlorophyll *a* cell^{-1} (Fig. 5) did not differ significantly between treatments at any time point under visible or UV light. β-carotene cell^{-1} was not significantly different between treatments, with the exception that control cultures had higher levels in visible light than in UV light at T_{12} (Fig. 6). The ratio of β-carotene to chlorophyll *a* did not differ with treatment (data not shown). Under visible light, fluoranthene addition had no significant effect on violaxanthin cell^{-1}, but under UV light, the violaxanthin content of control and acetone cultures was significantly higher than that of fluoranthene-treated cultures at T_{36} (Fig. 7). Similar to the patterns in violaxanthin content, the cellular concentration of zeaxanthin did not differ with treatment under visible light, but the UV + fluoranthene treatment resulted in significantly greater zeaxanthin cell^{-1} than that in the control or acetone treatments at T_{24} (Fig. 8). The ratio of zeaxanthin to

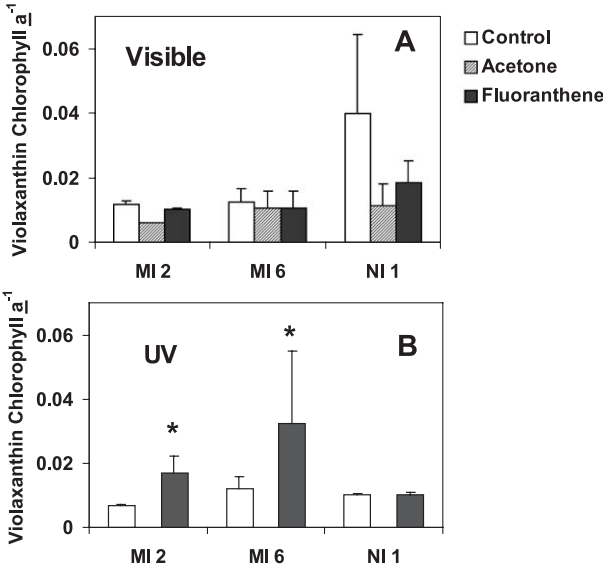

Fig. 12. Mean and standard deviation (error bars) of violaxanthin chlorophyll a^{-1} in water samples collected from Murrells Inlet sites 2 (MI 2) or 6 (MI 6), or North Inlet site 1 (NI 1), and incubated with (A) no additions (Control), acetone, or fluoranthene under visible light or (B) no additions (Control), or fluoranthene under UV light. * indicates significant difference between Fluroanthene and Control treatments.

violaxanthin did not change significantly with treatment under visible light, but was significantly increased by fluoranthene addition under UV light (e.g. by >10-fold at T_{36}; Fig. 9).

3.2. Natural community experiment

Chlorophyll *a* concentrations were not significantly affected by fluoranthene addition under visible light (Fig. 10A), or by UV vs. visible light ("controls" in Fig. 10). However, chlorophyll *a* levels under UV + fluoranthene were significantly lower in Murrells Inlet samples (MI 2, MI 6), but not North Inlet samples (NI 1); Fig. 10B. The ratio of carotene to chlorophyll *a* in control cultures was significantly higher under visible light than UV light in the natural community samples (Fig. 11). This ratio did not differ significantly between treatments under visible light (Fig. 11A), but under UV light, the ratio was significantly higher in UV + fluoranthene treatments from the MI 2 and MI 6 sites (but not from NI 1) when compared to UV controls (Fig. 11B). Similarly, there was no significant difference in the violaxanthin to chlorophyll *a* (Fig. 12A) or zeaxanthin to chlorophyll *a* ratio (Fig. 13A) between treatments in visible light, but these ratios increased significantly in MI 2 and MI 6 (but not NI 1) samples under UV light when fluoranthene was added (Figs. 12B, 13B). The ratio of zeaxanthin to violaxanthin was also found to vary in the UV light treatments, but not under visible

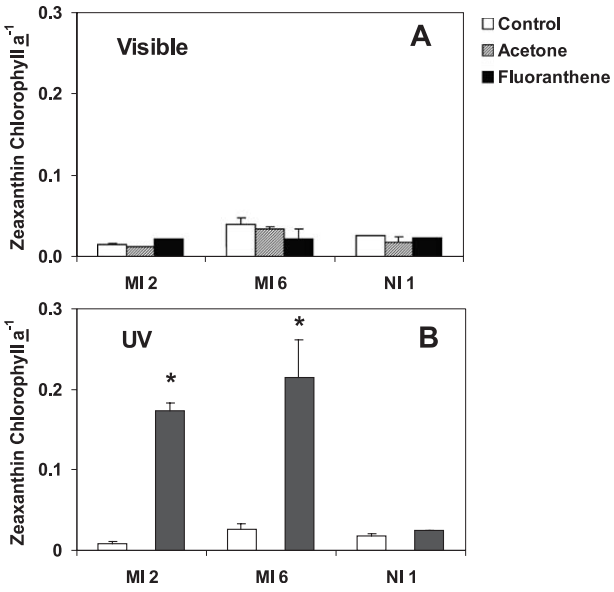

Fig. 13. Mean and standard deviation (error bars) of zeaxanthin chlorophyll a^{-1} in water samples collected from Murrells Inlet sites 2 (MI 2) or 6 (MI 6), or North Inlet site 1 (NI 1), and incubated with (A) no additions (Control), acetone, or fluoranthene under visible light or (B) no additions (Control), or fluoranthene under UV light. * indicates significant difference between Fluroanthene and Control treatments.

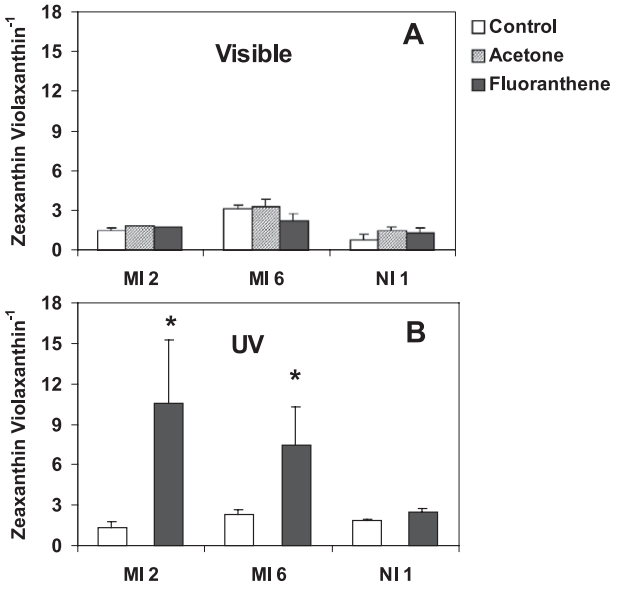

Fig. 14. Mean and standard deviation (error bars) of zeaxanthin violaxanthin^{-1} in water samples collected from Murrells Inlet sites 2 (MI 2) or 6 (MI 6), or North Inlet site 1 (NI 1), and incubated with (A) no additions (Control), acetone, or fluoranthene under visible light or (B) no additions (Control), or fluoranthene under UV light. * indicates significant difference between Fluroanthene and Control treatments.

light, with significantly higher ratios measured in fluoranthene-treated samples from MI2 and MI6, but not NI 1 (Fig. 14).

4. Discussion

The UV-A intensity tested (800 μW cm^{-2}) compares favorably with intensities observed in Murrells Inlet estuary. A study by Williamson (2000) reported that mean surface UV-A intensities ranged from 675 to 2683 μW cm^{-2}, with lowest intensities occurring in the winter months and greatest intensities occurring in the spring and summer. However, light intensities measured at the surface can be substantially reduced at the sediment–water interface due to light attenuation. The same study developed a spatial model which predicted the percentage of the area of Murrells Inlet sediments subjected to a UV-A intensity of at least 581 μW cm^{-2} at low tide during the months of October 1998 (62%), May 1999 (63%), and August 1999 (63%). Based on these data, the intensity tested in this experiment is within the range that may be expected to exist throughout a substantial portion of the water column and sediments. This UV level is apparently not sufficient to induce photoinhibition in *Ankistrodesmus*, as there was no significant difference in cell abundance or chlorophyll *a* production between UV and visible light controls.

The fluoranthene concentrations tested, 19 μg l^{-1} (*Ankistrodesmus* experiments) or 95 μg l^{-1} (natural community experiments) are 2–3 orders of magnitude higher than the mean water column fluoranthene concentration observed by one study of Murrells Inlet runoff events, 0.13 μg l^{-1} (Siewicki, 1995). This may indicate that Murrells Inlet phytoplankton communities are susceptible to fluoranthene toxicity in the water column only during extreme runoff events, or in heavily polluted areas. However, fluoranthene readily sorbs to particles, and sediment accumulation can be far greater than that in the water column (Chandler et al., 1997; Siewicki, 1997). Benthic algal species, such as *Ankistrodesmus*, may be particularly at risk to UV-induced fluoranthene toxicity due to direct contact with the sediments.

We tested the physiological response of *Ankistrodesmus* to fluoranthene addition at 19 μ l^{-1}, and did not observe a significant effect on growth rate, but did see consistent patterns in pigment composition. The lack of an effect of UV + fluoranthene treatment on β-carotene $cell^{-1}$ in *Ankistrodesmus* cultures does not support the hypothesis that cellular content of this pigment increases in response to UV-activated fluoranthene toxicity, and suggests that physiological mechanisms for protection against oxygen radical damage may differ between this species and *S. capricornutum* (Gala and Giesy, 1993). In contrast, effects of this PAH on cellular concentrations of zeaxanthin (increase) and violaxanthin (decrease), and the resultant increase in the ratio of zeaxanthin/violaxanthin, are consistent with the hypothesis that *Ankistrodesmus* responds to UV-activated fluoranthene toxicity by xanthophyll cycle activity.

The natural community bioassays were conducted to test whether pigment composition patterns were at least consistent with either or both of the hypothesized phytoplankton physiological responses to UV-activated fluoranthene toxicity. In contrast to the previous laboratory results using *Ankistrodesmus*, the carotene/chlorophyll *a* ratio decreased in response to UV control samples from both MI locations, but increased in UV + fluoranthene treatments from those sites. Either this latter treatment selected for the growth of phytoplankton with relatively high carotene chlorophyll a^{-1} or the treatment affected the carotene content of some portion of the ambient phytoplankton community (i.e. in contrast to the observed responses of *Ankistrodesmus*).

The ratio of zeaxanthin/chlorophyll *a* was elevated by the UV + fluoranthene treatment in water collected from the MI 2 and MI 6 sites, while the violaxanthin/chlorophyll *a* ratio was reduced by this treatment. Also, a significantly higher mean zeaxanthin/violaxanthin ratio was observed in the UV + fluoranthene treatment in MI 6 samples, and was nearly an order of magnitude higher in MI 2 samples. In contrast, no significant response was observed in the UV + fluoranthene treated samples from NI. Mean sediment fluoranthene concentrations reported for MI 2 were higher than those reported for MI 6 (Siewicki, 1995), and mean sediment PAH concentrations were higher in MI than NI (Fortner et al., 1996). If these responses reflect cellular pigment shifts and not community composition changes, the increase in the zeaxanthin/violaxanthin ratio in samples from the more heavily impacted sites may indicate that algae from these sites have developed more rapid physiological response capabilities to UV-activated fluoranthene toxicity and/or a lower threshold for induction of the xanthophyll cycle as a result of chronic exposure to PAHs. Alternatively, this might suggest that phytoplankton from the heavily impacted sites were physiologically stressed initially and more susceptible to UV-induced fluoranthene toxicity

than those from the less impacted site. Although the lack of phytoplankton taxonomic information precludes our ability to differentiate between pigment changes related to community shifts vs. cellular physiology, these results are at least consistent with xanthophyll cycling in MI phytoplankton communities.

5. Conclusions and future directions

The goal of this study was to test hypotheses related to photopigment responses of a benthic green alga to UV-induced fluoranthene toxicity. Contrary to one hypothesis, β-carotene was not observed to increase in response to UV-activated fluoranthene toxicity in *Ankistrodesmus* sp. However, the ratio of zeaxanthin/violaxanthin was shown to increase in UV + fluoranthene treatments, consistent with the hypothesis that xanthophyll cycling is a photophysiological response of this alga to UV-induced fluoranthene toxicity. Confirmatory tests of the hypothesis are required that include examination of the dynamical relationship of antheraxanthin and enzymatic measurements of xanthophyll cycle pathways. If confirmed, research into the relative importance of this response to cellular physiological functioning should include comparative studies on alternative photophysiological responses to oxidative stress; e.g. respiratory pathways (Lewitus and Kana, 1995; Eriksen and Lewitus, 1999, designated as *Closterium* sp.).

The carotene/chlorophyll *a*, zeaxanthin/chlorophyll *a*, and zeaxanthin/violaxanthin ratios increased in natural phytoplankton communities (MI 2 and MI 6) samples that were exposed to UV + fluoranthene. These responses are consistent with both hypotheses, and suggest that both pigment responses may occur in nature (increase in protective carotenoids such as β-carotene and cycling to carotenoids with lower energy states, e.g. zeaxanthin). However, several alternative interpretations of pigment responses are possible, and taxonomic assessments of community composition are critical for resolution. The natural community responses indicated that pigment composition was more responsive in the estuary with higher ambient PAH levels (Murrells Inlet). The degree to which these patterns reflect changes in cellular physiology and species composition has implications to understanding the effects of PAH loading (e.g. chronic PAH exposure) on estuarine microalgal productivity.

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References

Bastian, M.V., Toetz, D.W., 1982. Effect of eight polynuclear aromatic hydrocarbons on growth of *Anaebena flos-aquae*. Bull. Environ. Contam. Toxicol. 29, 531–538.

Boese, B.L., Lamberson, J.O., Swartz, R.C., Ozretich, R., Cole, F., 1998. Photoinduced toxicity of PAHs and alkylated PAHs to a marine infaunal amphipod (*Rhepoxynius abronius*). Arch. Environ. Contam. Toxicol. 34, 235–240.

Brand, L.E., Guillard, R.R.L., Murphy, L.S., 1981. A method for the rapid and precise determination of acclimated phytoplankton reproduction rates. J. Plankton Res. 3, 193–201.

Chandler, G.T., Shipp, M.R., Donelan, T.L., 1997. Bioaccumulation, growth and larval settlement effects of sediment associated polynuclear aromatic hydrocarbons on the estuarine polycheate, *Streblospio benedicti*. J. Exp. Mar. Biol. Ecol. 213, 95–110.

Eriksen, N.T., Lewitus, A.J., 1999. Cyanide-resistant respiration in diverse marine phytoplankton. Evdience for the widespread occurrence of the alternative oxidase. Aquat. Microb. Ecol. 17, 145–152.

Falkowski, P.G., Raven, J.A., 1997. Aquatic Photosynthesis. Blackwell Science, Malden, MA.

Fortner, A.R., Sanders, M., Sharon, W.L., 1996. Polynuclear aromatic hydrocarbon and trace metal burdens in sediment and the oyster, *Crassostrea virginica* Gmelin, from two high-salinity estuaries in South Carolina. In: Vernberg, F.J., Vernberg, W.B., Siewicki, T. (Eds.), Sustainable Development in the Southeastern Coastal Zone. University of South Carolina Press, Columbia, SC, pp. 445–475.

Gala, W.R., Giesy, J.P., 1992. Photoinduced toxicity of anthracene to the green algae *Selenastrum capricornutum*. Arch. Environ. Contam. Toxicol. 23, 316–323.

Gala, W.R., Giesy, J.P., 1993. Using the carotenoid biosynthesis inhibiting herbicide, Fluoridone, to investigate the ability of carotenoid pigments to protect algae from the photo induced toxicity of anthracene. Aquat. Toxicol. 27, 61–70.

Gala, W.R., Giesy, J.P., 1994. Flow cytometric determination of the photoinduced toxicity of anthracene to the green algae *Selenastrum capricornutum*. Environ. Toxicol. Chem. 13, 831–840.

Goodwin, T.W., 1980. The Biochemistry of the Carotenoids, 2nd ed. Plants, vol. I. Chapman & Hall, New York.

Guillard, R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chaney, M.H. (Eds.), Culture of Marine Invertebrate Animals. Plenum, New York, pp. 29–60.

Hamilton, M.A., Russo, R.C., Thurston, R.V., 1977. Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11, 714–719.

Ireland, D.S., Burton, G.A., 1996. In situ toxicity evaluations of turbidity and photoinduction of polycyclic aromatic hydrocarbons. Environ. Toxicol. Chem. 15, 574–581.

Kleinbaum, D.G., Kulpepper, K.L., Muller, K.E., Nizam, A., 1998. Applied Regression Analysis and other Multivariate Methods, 3rd ed. Duxbury Press, Pacific Grove, CA.

Lewitus, A.J., Kana, T.M., 1994. Responses of estuarine phytoplankton to exogenous glucose: stimulation versus inhibition of photosynthesis and respiration. Limnol. Oceanogr. 39, 182–189.

Lewitus, A.J., Kana, T.M., 1995. Light respiration in six estuarine phytoplankton species: contrasts under photoautotrophic and mixotrophic growth conditions. J. Phycol. 31, 754–761.

Lewitus, A.J., Koepfler, E.T., Morris, J.T., 1998. Seasonal variation in the regulation of phytoplankton by nitrogen and grazing in a salt marsh estuary. Limnol. Oceanogr. 43, 636–646.

Lotufo, G.R., 1998. Lethal and sublethal toxicity of sediment associated fluoranthene to benthic copepods: application of the critical body residue. Aquat. Toxicol. 44, 17–30.

Porra, R.J., Pfundel, E.E., Engel, N., 1997. Metabolism and function of photosynthetic pigments. In: Jeffrey, S.W., Mantoura, R.F.C., Wright, S.W. (Eds.), Phytoplankton Pigments in Oceanography. UNESCO, Paris, pp. 85–126.

Ren, L., Zeiler, L.F., Dixon, G.D., Greenberg, B.M., 1996. Photoinduced effects of polyaromatic hydrocarbons on *Brassica napus* (Canola) during germination and early seed development. Ecotoxicol. Environ. Saf. 33, 73–78.

Sall, J., Lehman, H., 1996. JMP® Start Statistics: A Guide to Statistics and Data Analysis Using JMP® and JMP IN® Software. SAS Institute, Cary, NC.

Siewicki, T.C., 1995. PhD Dissertation. Bioaccumulation of sediment associated fluoranthene by eastern oysters

(*Crassoteria virginica*): kinetics, environmental fate, statistical and environmental monitoring and exposure assessment. University of South Carolina Press, Columbia, SC.

Siewicki, T.C., 1997. Environmental modeling and exposure assessment of sediment-associated fluoranthene in a small, urbanized, non-riverine estuary. J. Exp. Mar. Biol. Ecol. 213, 71–94.

Southerland, H.A., 2001. MS Dissertation. Physiological responses of estuarine phytoplankton to photoinduced fluoranthene toxicity. University of South Carolina Press, Columbia, SC.

Van Heukelem, L., Lewitus, A.J., Kana, T.M., Craft, N.E., 1992. High performance liquid chromatography of phytoplankton pigments using a polymeric reversed-phase C_{18} column. J. Phycol. 28, 867–872.

Van Heukelem, L., Lewitus, A.J., Kana, T.M., Craft, N.E., 1994. Improved separations of phytoplankton pigments using temperature-controlled high performance liquid chromatography. Mar. Ecol. Prog. Ser. 114, 303–313.

Vernberg, F.J., Vernberg, W.B., Blood, E., Fortner, A., Fulton, M., McKellar, H.N., Michenar, W., Scott, G., Siewicki, T., Figi, K.E., 1992. Impact of urbanization on high-salinity estuaries in the southeastern United States. Neth. J. Sea Res. 30, 239–248.

Weinstein, J.E., Oris, J.T., Taylor, D.H., 1997. An ultrastructural examination of the mode of UV-induced toxic action of fluoranthene in the fathead minnow, *Pimphales promelas*. Aquat. Toxicol. 39, 1–22.

Williamson, M.W., 2000. MS Thesis. Integrating meiobenthic copepod bioassays and spatial modeling techniques to enhanced photo-enhanced toxicological hazard of Murrells Inlet sediments contaminated with polycyclic aromatic hydrocarbons. University of South Carolina Press, Columbia, SC.